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The Germination Of The Pollen Of

Tropaeolum Majus, L., And Lathyrus Odorata, L.

THE GERMINATION OF THE POLLEN OF TROPAEOLUM MAJUS, L., AND LATHYRUS ODORATA, L.

BY

ROSE SMITH

A. B. University of Illinois, 1911

THESIS

Submitted in Partial Fulfillment of the Requirements for the

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Rose Smith

ENTITLED The Germination of the Pollen of Tropaeolum Majus, L., and Lathyrus Odorata, L.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Arts

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THE GERMINATION OF THE POLLEN OF TROPAEOLUM MAJUS, L.,

AND LATHYRUS ODORATA, L.

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THE GERMINATION OF THE POLLEN OF TROPAEOLUM MAJUS, L., AND LATHYRUS ODORATA, L.

I Introduction

During the summer of 1915 in the botanical laboratory at the University of Illinois, the data for this article were collected under the direction of Dr. C. F. Hottes. The intention was to study the pollen of a number of summer flowers, but weather conditions limited me to a more detailed study of the pollen of Tropaeolum majus and Lathyrus odorata. Attention was given more particularly to the factors that determined the number of grains germinated, and the rate of growth of the tubes. Since the grains ordinarily germinate on the stigma moistened by a secretion of varying osmotic pressure, a study was made of the influence of solutions of different osmotic power on the germination of the grains and the rate of growth of the pollen tubes.

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II Historical

Jost ('05) divided the pollen with which he experimented into three divisions: that which required water for germination; that which would germinate in water plus some chemical, as an acid; and that which germinated only in certain concentrations of sugar. Pfundt ('10) found that the pollen of many flowers germinated in cane sugar solutions varying in concentration from one to fifty per cent. The average for angiosperms was from thirty to forty per cent. The optimum for graminae was from fifteen to twenty per cent. Martin ('13) experimented with three of the clovers in different concentrations of sugar. His results showed that Trifolium pratense did not germinate in the presence of sugar solutions while Trifolium hydridium and Trifolium repens did; and that it was the sugar which controlled the water supply in the last two. Lidforss ('96) paid chief attention to the second division of Jost, that is, to the effects of small quantities of certain mineral salts on the germination of some kinds of pollen. His studies showed that the more concentrated solutions brought about the death of the pollen. Later he (Lidforss, '99) continued these experiments and found that all pollen except that of the xerophytes germinated better in moist than in dry air. The observations of Molisch ('93) showed that most pollen germinated in cane sugar solutions from one to fifty percent. The life of the pollen tube varied, the longest being

seventy-two days. When in his studies he placed the pollen of Viola hinta in gelatine with a mature pistil, pollen tubes grew chematropically toward the pistil. Miyoshi ('94) found that the pollen tubes were positively attracted toward the stigma and concluded that it was due to the moisture and the small quantity of cane sugar which the stigma contained. Davenport ('97-'99 and '08) stated that the germination of pollen demanded a certain minimum of water; that this quantity varied with different plants; and that the direction of growth was influenced by hydrotropism.

III Materials and Methods

The pollens of Lathyrus odorata and Tropaeolum majus were used. The material was collected in the morning and immediately used.

The only way, according to Livingston, to compare different media is by their osmotic pressure.[1] Since osmotic pressure varies directly with the number of molecules in the solution, the gram molecule solution as well as the per cent solution was computed. The gram molecule solution was determined from the per cent solution according to the formulae given by Renner.[2]

The solutions were prepared on the percentage basis. To each was added one and one half parts of gelatine. In all the media prepared the quantity of gelatine was kept constant. The reason for using gelatine was to make the hanging drop slightly viscid.

Germination was studied by the hanging drop method in Van Tiegham cells.

The flowers used were collected about eight o'clock in the morning and brought into the laboratory. The ripe anthers were lightly touched with a blunt dissecting needle and the pollen adhering was placed in a drop of the medium on the cover glass

[1]Livingston, The Rôle of Diffusion and Osmotic Pressure in Plants, 6.

[2]Renner, Über die Berechmung des osmotischen Druckes, 11.

which was then inverted over the Van Tiegham cell. The whole was placed on a plaster block in a Koch moist chamber with sufficient water to cover the plaster block to half its depth.

After the germination had been determined by this method, the process was slightly altered. A fresh stigma was pressed on the cover glass before placing the drop of the medium upon it; then a drop of the solution was added and the pollen was placed around the stigma. The purpose was to determine whether the stigma exerted an influence on pollen germination.

As a final test to ascertain whether the quantity of water present was the factor which determined the germination of the pollen, the manner of procedure was again changed. Centimeter squares of gelatine were placed in the aqueous medium for five minutes. They were then removed, superficially dried with filter paper, placed on glass slides, the pollen applied, and the slides placed in the Koch moist chambers.

All experiments were carried on at room temperature, in moderate light, but never in direct sunlight. The results were recorded after the pollen had been in the medium three hours. The data that follow are the average of repeated experiments.

IV Experiments and Discussions

Action of different media upon pollen

Table I

Results obtained when pollen was placed in sugar media

Lathyrus Odorata

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germination	Per cent pseudo germination	Per cent of bursting	Aver. tube length in mm.
Cane sugar	.2926	10	33	15	0	31	.0666
" "	.3216	11	45	20	0	28	
" "	.3508	12	38	35	0	30	
" "	.3801	13	26	30	0	15	.1777
" "	.4093	14	30	53	0	0	
" "	.4385	15	18	66	0	0	
" "	.4678	16	73	84	0	0	.1777
" "	.4970	17	40	75	0	0	
" "	.5263	18	32	34	0	0	
" "	.5555	19	46	26	0	0	
" "	.5847	20	34	26	0	0	.1554
" "	.6140	21	56	9	0	0	
" "	.6432	22	50	2	0	0	
" "	.6725	23	30	6	0	0	.1110
" "	.7017	24	40	0	0	0	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Cane sugar	.7309	25	42	0	0	0	
" "	.7600	26	45	0	0	0	
" "	.7891	27	38	0	0	0	
" "	.8182	28	43	0	0	0	
" "	.8473	29	47	0	0	0	
" "	.8765	30	42	0	0	0	
" "	.9056	31	44	0	0	0	
" "	.9348	32	39	0	0	0	
" "	.9639	33	41	0	0	0	
" "	.9941	34	46	0	0	0	
" "	1.0233	35	37	0	0	0	
" "	1.0524	36	40	0	0	0	
" "	1.0815	37	51	0	0	0	
Dextrose	.5555	10	60	6	0	31	
"	.6111	11	55	8	0	38	
"	.6666	12	87	10	0	32	.1998
"	.7222	13	87	12	0	36	
"	.7777	14	65	23	0	36	
"	.8333	15	30	20	0	50	
"	.8888	16	32	30	0	53	.0888
"	.9444	17	35	34	0	51	
"	1.0000	18	42	28	0	17	.0832
"	1.0555	19	40	10	0	0	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Dextrose	1.1111	20	26	0	0	0	
"	1.1666	21	33	3	0	0	.0555
"	1.2222	22	38	0	0	0	
"	1.2777	23	39	0	0	0	
"	1.3333	24	42	0	0	0	
"	1.3888	25	41	0	0	0	
"	1.4444	26	35	0	0	0	
"	1.5000	27	43	0	0	0	
"	1.5555	28	37	0	0	0	
Lactose	.1388	5	71	7	0	14	
"	.1664	6	86	10	0	9	
"	.1940	7	92	4	0	5	
"	.2216	8	76	5	0	4	
"	.2492	9	79	8	0	5	
"	.2776	10	96	10	0	4	
"	.3052	11	129	8	1	0	.5110
"	.3328	12	104	9	0	0	
"	.3604	13	93	12	1	0	
"	.3880	14	146	9	2	0	
"	.4166	15	80	10	0	0	.4444
"	.4444	16	101	22	0	2	
"	.4720	17	95	28	0	1	
"	.4976	18	103	37	0	0	.4444

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Dextrine	.3086	5	84	7	0	35	.6105
"	.3703	6	84	12	0	23	
"	.4320	7	65	11	0	33	
"	.4938	8	76	13	0	22	
"	.5555	9	66	34	0	23	
"	.6172	10	81	42	0	21	.2775
"	.6790	11	80	58	0	21	
"	.7387	12	90	50	0	16	
"	.8005	13	88	57	0	16	.2553
"	.8623	14	80	50	0	22	
"	.9241	15	78	57	0	21	
"	.9858	16	81	74	0	17	.2664
"	1.0475	17	92	42	0	28	
"	1.1092	18	89	25	0	29	.2332
"	1.1710	19	80	38	0	35	
"	1.2327	20	81	37	0	30	
"	1.2962	21	85	28	0	25	
"	1.3579	22	87	16	0	22	.0888
Mannit	.2801	5	92	0	0	51	
"	.3296	6	91	0	0	36	
"	.3846	7	96	0	0	23	
"	.4395	8	99	0	0	22	
"	.4944	9	100	0	1	13	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Mannit	.5494	10	96	0	3	15	
"	.6043	11	86	1	0	15	
"	.6593	12	86	1	0	9	
"	.7142	13	89	2	1	7	
"	.7692	14	99	3	0	6	
"	.8247	15	110	3	0	3	
"	.8791	16	90	4	0	2	.1665
"	.9340	17	80	1	0	0	
Distilled water			66	2	0	58	
Tap water			90	1	0	55	

Tropaeolum Majus

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germination	Per cent pseudo germination	Per cent of bursting	Aver. tube length in mm.
Cane sugar							
" "	.2926	10	34	0	22	66	
" "	.3216	11	38	0	20	33	
" "	.3508	12	27	0	24	25	
" "	.3801	13	63	0	3	34	
" "	.4093	14	41	0	9	41	
" "	.4385	15	33	0	3	40	
" "	.4678	16	49	0	6	71	
" "	.4970	17	50	4	0	80	.2386
" "	.5263	18	55	6	12	70	
" "	.5555	19	33	3	80	15	
" "	.5847	20	27	7	81	0	
" "	.6140	21	50	4	92	0	
" "	.6432	22	47	2	94	0	.1443
" "	.6725	23	63	5	85	0	
" "	.7017	24	54	7	73	0	
" "	.7309	25	53	8	84	0	
" "	.7600	26	40	20	75	0	
" "	.7891	27	35	20	74	0	
" "	.8182	28	36	33	55	0	.0850
" "	.8473	29	42	67	23	0	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Cane sugar	.8765	30	50	90	2	0	
" "	.9056	31	31	90	0	0	.0777
" "	.9348	32	44	75	8	0	
" "	.9639	33	45	73	9	0	
" "	.9941	34	44	20	6	0	
" "	1.0233	35	60	11	0	0	.0333
" "	1.0524	36	52	0	0	0	
" "	1.0815	37	49	0	0	0	
Dextrose	.5555	10	108	0	0	39	
"	.6111	11	104	0	0	27	
"	.6666	12	95	0	0	42	
"	.7222	13	115	0	0	38	
"	.7777	14	119	0	2	53	
"	.8333	15	110	0	7	42	
"	.8888	16	141	1	0	42	
"	.9444	17	112	10	0	41	.1443
"	1.0000	18	50	14	0	6	
"	1.0555	19	99	11	0	3	
"	1.1111	20	105	27	3	12	
"	1.1666	21	69	33	1	3	.0888
"	1.2222	22	80	16	0	0	
"	1.2777	23	43	15	0	0	.0722
"	1.3333	24	109	27	0	0	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Dextrose	1.3888	25	62	0	0	0	
"	1.4444	26	51	0	0	12	
"	1.5000	27	53	0	0	3	
"	1.5555	28	58	0	0	0	
Lactose	.1388	5	83	0	0	54	
"	.1664	6	84	0	0	50	
"	.1940	7	80	0	0	43	
"	.2216	8	96	0	0	42	
"	.2492	9	83	0	0	36	
"	.2776	10	85	0	0	36	
"	.3052	11	120	0	0	25	
"	.3328	12	79	3	0	29	.2444
"	.3604	13	98	3	0	29	
"	.3880	14	110	3	0	23	
"	.4166	15	95	4	0	25	.1999
"	.4444	16	91	4	0	21	
"	.4720	17	103	5	0	21	
"	.4976	18	100	7	0	17	.1665
Dextrine	.3086	5	86	0	0	33	
"	.3703	6	83	0	0	30	
"	.4320	7	80	0	0	30	
"	.4938	8	80	0	0	25	
"	.5555	9	75	0	1	20	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Dextrine	.6172	10	83	0	1	17	
"	.6790	11	76	0	3	19	
"	.7387	12	80	0	10	15	
"	.8005	13	82	0	9	17	
"	.8623	14	82	2	11	27	
"	.9241	15	50	3	16	28	.0721
"	.9858	16	85	4	12	17	
"	1.0475	17	88	4	12	16	
"	1.1092	18	82	3	8	23	
"	1.1710	19	86	7	9	16	
"	1.2327	20	90	7	5	15	.0666
"	1.2962	21	89	3	4	16	
"	1.3579	22	87	6	3	14	.0555
Mannit	.2801	5	90	0	0	28	
"	.3296	6	97	0	0	20	
"	.3846	7	94	0	0	23	
"	.4395	8	91	0	0	23	
"	.4944	9	93	0	0	19	
"	.5494	10	87	0	0	13	
"	.6043	11	91	0	0	10	
"	.6593	12	86	0	0	13	
"	.7142	13	90	0	0	20	
"	.7692	14	87	0	0	16	

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Mannit	.8247	15	75	0	0	10	
"	.8791	16	81	0	0	9	
"	.9340	17	83	0	0	8	
Distilled water			80	0	0	70	
Tap water			140	0	0	79	

Within five minutes after the pollen of Tropaeolum majus was placed in a hanging drop of tap water seventy per cent of the grains burst; with Lathyrus odorata the action was even quicker, but the per cent of bursting was lower. In some trials with Lathyrus odorata a few grains germinated; but with Tropaeolum majus none. The results obtained with distilled water were about the same.

The pollen of Lathyrus odorata germinated in all the media used. Tropaeolum majus germinated in all media except mannit. In lactose the per cent of germination was low.

Table I shows that the pollens of Lathyrus odorata and of Tropaeolum majus burst in distilled water and in tap water; that the bursting lessens in low concentrations of sugar; that bursting ceases with high concentrations, unless the substance crystallizes at a low concentration.

A comparison of the optimum germination of the pollen of

Lathyrus odorata in the different media shows that it occurred in widely different gram molecule solutions. With cane sugar it took place in a .4678 gram molecule solution; with lactose, .4996; with dextrose, .9944; with dextrine, .9858; and with mannit, .9340. Tropaeolum majus showed the same variation. Evidently, osmotic pressure did not determine the germination of the pollen grains.

Influence of stigma on pollen germination

Table II

Results obtained when stigma was placed with pollen in hanging drop

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent of burst-ing
Lathyrus Odorata					
Cane sugar	.4678	16	52	65	11
" "	.9064	30	56	0	0
Dextrose	.9444	17	42	15	42
"	1.1666	21	50	6	0
Lactose	.5000	18	40	27	21
Dextrine	1.0493	17	58	37	8
Mannit	.9890	17	50	0	18
Tropaeolum Majus					
Cane sugar	.4678	16	68	0	79
" "	.9064	30	50	52	0
Dextrose	.9444	17	50	0	0
"	1.1666	21	53	26	0
Lactose	.5000	18	50	0	16
Dextrine	1.0493	17	72	6	43
Mannit	.9890	17	50	0	20

In determining whether the stigma exerted an influence on pollen germination only such concentrations of sugar solution

were used as showed an optimum in Tropaeolum majus and Lathyrus odorata, Table I. In Table II pseudo germination and bursting were placed together under the heading "bursting."

With the pollen distributed about the style and the stigma there was no indication that either exerted an influence on pollen tube formation. In fact, pollens some distance from the stigma germinated as well, and in some cases even better, than that nearer it. Neither did the stigma exert an influence on the direction of the pollen tubes, for, when pollen grains were placed on the stigma, the tubes turning away from the stigma were about as numerous as those turning towards it.

The data in Table II show that the germination was lower when the pollen alone was placed in the hanging drop. This may be accounted for, in that, either the stigma absorbed water from the medium, thus increasing the concentration; or that the atmospheric conditions at the time of the experiment were unfavorable. The pollen was collected during a series of days of cold rain. It is probable that at the concentration of sugar used in these experiments, the chemotropic influence, if any, of the stigma was counteracted. Also, it is possible that the changes occurring in the dying tissue of the stigma liberated material unfavorable to the germination of the pollen.

Pollen germinated on squares of gelatine

Table III

Results obtained when pollen was germinated on gelatine

Lathyrus Odorata

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains	Per cent germination	Per cent pseudo germination	Per cent of bursting	Aver. tube length in mm.
Cane sugar	.2926	10	94	85	0	0	
" "	.4678	16	100	88	0	0	
" "	.6725	23	100	84	0	0	.099
" "	.9056	31	112	87	0	0	.033
" "	1.0233	35	87	86	0	0	.022
Dextrose	.5555	10	82	88	0	0	.099
"	.8888	16	94	82	0	0	.066
"	1.0000	18	88	80	0	0	.066
"	1.2777	23	100	88	0	0	.033
"	1.5000	27	100	90	0	0	.011
Lactose	.1388	5	75	82	0	0	.462
"	.2776	10	90	85	0	0	.660
"	.4166	15	120	90	0	0	.605
"	.4996	18	96	91	0	0	.330
Dextrine	.3086	5	64	78	0	0	.330
"	.6172	10	88	73	0	0	
"	..9858	16	106	84	11	0	.242
"	1.3579	22	96	62	6	0	.055

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germination	Per cent pseudo germination	Per cent of bursting	Aver. tube length in mm.
Mannit	.2801	5	88	90	0	0	.330
"	.6593	12	94	91	0	0	.275
"	.9340	17	94	91	0	0	.132
Distilled water	.		50	78	6	0	.5555
Tap water			61	78	3	0	.5555
			Tropaeolum Majus				
Cane sugar	.2926	10	94	91	1	0	.330
" "	.4678	16	64	81	0	0	.275
" "	.6725	23	106	80	5	0	
" "	.9056	31	99	83	3	0	
" "	1.0233	35	60	80	0	0	.038
Dextrose	.5555	10	82	64	14	0	.352
"	.8888	16	76	89	0	0	.275
"	1.0000	18	100	84	0	0	.187
"	1.2777	23	94	33	0	0	.036
"	1.5000	27	98	14	0	0	.022
Lactose	.1388	5	75	84	0	0	.462
"	.2776	10	90	74	0	0	.660
"	.4166	15	60	90	1	0	.605
"	.4996	18	96	83	0	0	.330
Dextrine	.3086	5	82	48	24	0	.385
"	.6172	10	84	28	45	0	.484

Medium	Gram molecule solution	Per cent of sugar	Aver. no. of grains used	Per cent germi-nation	Per cent pseudo germi-nation	Per cent of burst-ing	Aver. tube length in mm.
Dextrine	.9858	16	90	42	13	0	.330
"	1.3579	22	86	69	0	0	.275
Mannit	.2801	5	50	0	64	0	
"	.6593	12	43	13	44	0	
"	.9340	17	47	6	32	0	
Distilled water			73	0	0	78	
Tap water			81	0	0	82	

With Lathyrus odorata, the results in Table I showed that nearly all grains burst when the pollen was placed in tap water. When pollen grains of the same plant were placed on gelatine soaked in tap water the germination was excellent; when the gelatine had been soaked in one of the media used in these experiments the germination was usually higher. As the concentration of the solution increased the length of the pollen tube usually diminished. With a twenty-seven per cent solution of dextrose the pollen tube appeared as a mere papilla.

With Tropaeolum majus the results differed, yet a comparison established the same facts. No germination occurred when the pollen was placed on gelatine soaked in tap water; considerable germination took place when the pollen grains were grown on gelatine soaked in one of the above media. Though the percentage of germination was

was not affected by the concentration of the solution, the length of the pollen tubes diminished as the concentration of the solution increased.

In Tables I and III these facts are brought out: That the pollen of both Lathyrus odorata and Tropaeolum majus require moisture for the formation of pollen tubes; that the amount of moisture required by each differs; that by limiting the amount of water, thus increasing the concentration of the solution, the germination is greater; that the dissolved substance does not of itself increase the germination, for with high and low concentrations the results are approximately the same; lastly, that the dissolved substance is not necessarily valuable as a food product.

Pseudo-germination

The bursting of pollen grains was considered under two headings: first, the term "pseudo-germination" was applied when the grain put out a tube that had a round, inclosed mass at its tip, or, when the tube was irregular at the tip and the contained granules frequently burst from it; second, the term "bursting" was applied when the granules were ejected into the solution by the bursting of the intine and exine of the pollen grain.

A comparison of the bursting and pseudo-germination of Tropaeolum majus in low concentrations of cane sugar, Table I, showed that the bursting was high. With higher concentrations the percentage of pseudo-germination was greater than that of bursting. With increasing concentrations, pseudo-germination gradu-

ally diminished until it ceased entirely. Before this concentration was reached, germination began.

The facts brought out by Table I in regard to pseudo-germination were that it did not occur in water, and that usually it was not found in very low concentrations of sugar. When the pollen was placed on gelatine soaked in water, bursting occurred. When the pollen was placed on gelatine soaked in different sugar solutions, no bursting occurred.

The fact that pseudo-germination and bursting occurred in water and in solutions containing a large per cent of water, and gradually diminished as the per cent of water was lessened in the medium shows that it is the amount of water which determines both pseudo-germination and bursting.

V Summary and Conclusion

1. The germination of Tropaeolum majus and Lathyrus odorata pollen is not dependent on the osmotic action of the medium as a stimulant.

2. The addition of the stigma to the medium does not increase germination.

3. Water is necessary for the formation of pollen tubes.

4. The pollen grains of Tropaeolum majus and Lathyrus odorata differ in the amount of water required for germination.

5. Lessening the amount of water, by dissolving substances in the water, may raise the percentage of germination.

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